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Recent Advances in Chemistry of Phytotoxins

by

Akitami ICHIHARA*

Phytotoxins are poisonous substances produced by plant pathogens that have adverse effects on plants. As a group, phytotoxins have no common structural features, and these belong to such diverse classes of compounds as acetogenin, peptide, terpenoid, steroid, alkaloid and combination of these classes.

The general features of phytotoxins isolated from plant pathogenic bacteria and fungi are briefly described. Isolation, biological activity, structure determination, synthesis, biosynthesis and utilization are mentioned on a bacterial toxin, coronatine, and fungal toxins, betaenones, altiloxins, solanapyrones and alboatrin in rather detail.

1. Foreword

Agricultural products contract diseases on account of various factors[1)]. The diseases occasioned by soil, weather conditions and such are called physiological diseases. They are not contagious. On the other hand the diseases caused by molds, microorganisms such as bacteria or by viral pathogens are called parasitic diseases. They are contagious. Parasitic diseases have often caused enormous damage in human history. It is said that the potato disease which afflicted Ireland in 1845 caused the death by starvation of one million people. Even today and in spite of all advances with regard to fungicides, one has heard the new fact that the US

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corn production dropped to one third in 1970 on account of the southern corn leaf blotch.

The microorganisms bringing about such blight produce all kinds of metabolites harmful to the host plants. The pathogenic bacteria inducing the soft rot condition in vegetables produce pectinesterase, and those which induce the rice leaf blotch disease release strong polyphenoloxidase which accelerates the damage caused by the disease itself. Besides such harmful enzymes, the micromolecular organic compounds which at a low physiological concentration cause harm to the host and to the other plants are called phytotoxins[2)]. From the vantage point of plant pathology the toxins are divided into host-specific toxins which exercise their harmful action only on the host plants[2,3)] and non-specific toxins which exercise their harmful action on the host plant and on the other plants at large[1,2)]. Since all presently known host-specific toxins originate from molds, the toxins produced by bacteria and most other molds are non-specific toxins.

Traditional studies on toxins have been pursued mainly from two standpoints. The first one is to investigate the role played by toxins in the pathogenicity of the pathogen with regard to the host plant. The question of the experimental proof determining how toxins are related to the damage demands extreme caution. However, since one may say about the numerous pathogens that only pathogenic fungi produce toxins, there is no doubt that toxins play an important part with regard to the blight of plants. The other standpoint is to seek in the phytopathogens a supply of physiological activators understood in a broad sense such as plant growth regulators. In this respect there are the five phytohormones viz. oxine, gibberellin, cytokinin, absidic acid and ethylene. It is interesting to note that all of them

are found as metabolites of phytopathogens. The recently noted phytohormone-like substances brassinolides have not yet been discovered as product of microorganisms.

In the present article we will present an overview of the metabolic toxins produced by phytopathogenic bacteria and molds and describe at the same time and more particularly the phytotoxins which we handled as well as the bioorganochemistry of their surrounding.

2. The toxins produced by the phytopathogenic bacteria p. 358

From the start of the present century it has been known that toxins played a part in the symptoms of the plant diseases caused by bacteria. However, the correct elucidation of their chemical structures dates only from the seventies. The bacterial toxins whose structure has been elucidated are the amino acids or their simple derivatives.

There is less diversification than among the mold toxins and their number is extremely small. The subject hereafter concerns a few phytopathogenic bacterial toxins whose chemistry has been studied.

2.1 Tabutoxin (1) This toxin is produced by Pseudomonas syringae pv. tabaci. The same toxin is also produced by Ps. syringae pv. coronafaciens and Ps. syringae pv. garcae[2]. The symptom is a formation of lesions with a yellow ring. In 1971 Stewart proposed the two-dimensional structural formula 1 for the structure of this toxin[4] (Fig. 1). This 1 is a very unstable compound. Under neutral conditions at room temperature it changes into isotabutoxin (2) and loses its activity. When 1 or 2 are hydrolyzed, they are induced into tabutoxin (3), tabutoxinin-δ-lactam (4), threonine (5). Through the synthesis of 2 performed by Rapoport et al in 1975, the relative

distribution of C-2, C-5 was demonstrated[5], and in 1983 the complete synthesis of 1 was reached by Baldwin et al[6]. There was a recent report on an improved method of synthesizing tabutoxinin-β-lactam[7].

2.2 Phaseolotoxin (7) This toxin is produced by Ps. syringae pv. phaseolicola and produces yellow lesions in the host leaves. The structure was first estimated to be 6[8] (Fig. 2). Recently it was corrected as 7 through FAB MS and ^{15}N-labelling tests[9]. However, 7 has not yet been synthesized and the three-dimensional phosphorus chemistry is unknown.

2.3 Tagetitoxin (8) The toxin is obtained from the culture solution of Ps. syringae pv. tagetis. At 20 ng, it produces spots. Because of detailed MS and ^{1}H NMR analyses, the specific hemithioketal structure (8) was presumed[10] but there is no X-ray crystallographic analysis or no chemical-synthesis-based confirmation.

2.4 Rhizobitoxin (9) This toxin is produced by Rhizobium japonicum. It forms yellowing spots on soya beans. Owen et al isolated it and determined the structure[11], but the estimation of the absolute configuration[12] and the chemical synthesis[13] were achieved by Keith et al.

2.5 Coronatine (10) It is a toxin produced by Ps. syringae pv. atropurpurea[14]. It is similarly produced by Ps. syringae pv. maculicola, Ps. syringae pv. morsprunorum[15], Ps. syringae pv. glycinea[16] (Fig. 3).

The present bacterium was treated for 3 days in an aerated culture with a 23°C artificially synthesized medium, and active coronatine (10) was isolated from the culture filtrate through various types of chromatography[17]. At the fractionation stage, one used a simple biological examination method,

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that is one indexed abnormal swelling with regard to potato tuper sections. p. 359 The molecular formula $C_{18}H_{25}O_4N$ was obtained through elemental analysis, high-resolution mass spectrum (HR-MS), and from two fragments we discovered that coronafassinic* acid (11a), $C_{12}H_{16}O_3$ and coronaminic* acid (12a), $C_6H_{11}O_2N$ perform amide bonding[17]. Even though 11a was separately isolated as the 2 three-dimensional isomers 11a, 11b from the culture solution, there was agreement with MS. Actually, by hydrolyzing 10 we confirmed the fact of spots matching 11a in chromatography. Meanwhile, when 10 is treated with acetic anhydride anhydrocoronatin (13) is produced. Since this IR exhibited typical azlactone absorption, we estimated 12a to be α-alkylamino acid. The structure of 11a was later ascertained by synthesis through X-ray crystallographic analysis. One of the 2 ethyl groups of 10 was present in the amino acid part. We considered the unsaturation degree,and formula 12 was presumed for this amino acid. Actually, a structure containing a relative configuration was ascertained through the synthesis of (±) -12a.

We determined the absolute configuration of 10 by first applying to the methylester of 11a the octant rule as well as by using optical rotatory dispersion (ORD) and circular dichroistic spectrum[18]. In order to determine the absolute configuration of 12a we synthesized all possible 4 isomers 12a-d as shown in Fig. 4[18,19]. We performed hydrogen degradation of the synthesized 12a and determined the absolute configuration of the degradation products by the enzyme method in which L- and D- amino acid oxidases are allowed

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*Translator's note: The * indicates that the spelling is not quite certain; it is the best educated guess for a term given in Japanese phonetics; same hereafter for * following the name of a particular chemical substance.

to act on the obtained products (Fig. 5) and by the X-ray crystallographic analysis of the acetate 12b[20]. When the synthesized amino acids (12a-d) and 11a were made to condense, the substance derived from 12a matched the natural substance, and therefore we determined that the absolute configuration of coronatine was 10[18,20]. We also used the 2-mode synthesis method for the synthesis of 11a[21] but will omit the explanations here as these were already given in the present journal[22]. After the synthesis of racemics[23,24] Nakamura and Ohira achieved recently the synthesis of the optically active coronafassinic* acid 11a[25]. We synthesized homologs in which the coronaminic* acid portion of 10 had been substituted to the three-dimensional isomers, amino acids, amino compounds and such of 12a, and examined the structure-activity correlation with regard to potato tubers. Carboxyl groups are essential for the manifestation of activity, and it became clear that the configuration (S) of the α-position plays an important role in the enhancement of activity[26].

Results interesting for the biosynthesis of coronatine were published recently[27]. When 1- and 2-^{13}C sodium acetate was added to the culture solution of these produced bacteria, the acetic acid of 5 molecules was incorporated. We also discovered that 1,2-^{13}C sodium pyruvate was incorporated as seen in Fig. 6. However, as no incorporation into C-3, C-3a was seen at this time, we assumed the fact of a new polyketide* route. We tested several precursors. In the end we discovered a biosynthesis through the branching polyketide route whereby the C-2, C-3 of 1,2,3-^{13}C pyruvic acid are incorporated. As for the synthesis of 12a, we discovered incorporation p. 360 into C-1' when adding a mixture of 1-^{13}C-DL-isolvicin* and 1-^{13}C-DL-aoisoloicin* to the medium.

At the coronatine research stage, it is the use of coronaminic acid (12a-d) which showed an unforeseen development. The 1-aminocyclopropane-1-carboxylic acid (14, ACC) is a phytohormone which plays a part in the maturation of fruits. It is a biosynthetic intermediate of ethylene (Fig. 7). Active research is being pursued about its production structure[28]. ACC itself is a prochiral compound without asymmetric carbon, and it should be extremely interesting to know whether or not a three-dimensional recognition takes place at the ethylene production process. The photoactive 12a-d corresponds to ACC labelled with an ethylene group and turned out to be a reasonable model. Since among the 4 three-dimensional isomers, only 12c is easily changed into 1-butane, the reaction model of 14 was proposed in the active location of the ethylene-producing enzyme[29] (Fig. 8). Another example concerns three-dimensional specificity at the time 12a only is induced to α-keto-n-caproic acid by ACC deaminase. From the section place of 12a, we saw that the C_1-pro S carbon of 14 is sectioned according to a three-dimensional specificity[30] (Fig. 9). Research in the synthesis of labelled compounds and isomers is actively being pursued in connection with these reactions of ACC[28].

3. Toxins produced by phytopathogenic molds

Among the phytopathogenic microorganisms, those produced by molds are overwhelmingly numerous. There are presently up to 8000 known types of fungus. Yet extremely few microorganisms have been the object of research in toxins. There are virtually no examinations of the fungi causing diseases in weeds, ornamental plants or trees. No common structural characteristics are seen in the phytotoxins originated from molds. Throughout the so-called

natural products such as acetogenin, terpene, peptide, steroid, alkaloid there is a difference from the metabolic toxins of the bacteria.

4. Host-specific toxins

Thirteen species of pathogenic molds produce 14 types of host-specific toxins. The chemical structure of 11 of these toxins has been explained. We will omit the details as these have been compiled in the form of a complete book and general report[3,3)]. Very recently one has explained the structure of the metabolic toxin AF-toxin category of the strawberry black-spot disease agent[31)]. It is strangely similar to the AK-toxin category[32)] isolated from the pear black-spot disease agent. It is interesting to note that the AF-toxin exhibits also a toxicity toward pears (nijisseiki).

5. Non-specific toxins

After invading the host, numerous phytopathogenic microorganisms metabolize all sorts of compounds which exercise a harmful action upon the host's cells. However, the non-specific toxins are not primary determining factors of disease. It is nevertheless believed that they play an important role in the process of the disease symptoms manifestation and of the increase in invading molds inside the tissues. These toxins do not only act as phytotoxins but are also often supplied as physiologically active substances. However, with the culture-based methods, they are not easily obtained quantitatively because of the mutations and such of the fungi. Synthetic chemical means are extremely effective for toxin supply which includes structure recognition and activity correlation. We will explain hereafter several phytotoxins whose structure was recently certified in our laboratory.

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5.1 Betaenones and aphidecholine* Phoma betae Fr. parasitizes the leaves, stems, seeds and the like of the sugar beet used to make sugar, and causes diseases. The symptom of the disease is the formation of red-brown-to-yellow concentric circular spots having a diameter of 1-2 cm.

We cultured this agent at 25°C for 15 days in a potato broth medium to which sugar had been added. We extracted the medium filtrate with ethyl-acetate and fractionated the extract with a silica gel column. As for the various fractions, we indexed sugar beet leaf activity tests, young lettuce plant growth inhibition tests, and isolated two groups of phytotoxin viz. the betaenones[33,34] and the aphidecholines*[35].

5.1.1 The betaenones Betaenone A (15) which has a new type of structure p. 361 is a crystal, and is low in toxicity. Betaenone B (16) was obtained in the largest quantity[33]. Betaenone C (17) has the highest activity. It was isolated in an oily form[34]. We also isolated successively the betaenones D (18), E (19), F (20) of which the quantities are small[34] (Fig. 10). As for the physiological activity, 17 was most actively inhibiting the growth of young sugar beet, lettuce, rice plants. As for the inhibiting activity in the synthesis of DNA, RNA and proteins with starfish gastrula, 17 almost completely inhibited the synthesis of RNA and of proteins at 20 μ/mL. Since the simultaneously isolated aphidecholine* inhibits specifically the DNA polymerase α, it seems that 17 displays its phytotoxicity through a different mechanism.

As the various spectra of the betaenones (15-20) show similar patterns, it seems that the basic structure is common. We first performed the X-ray crystallographic analysis and demonstrated a structure containing a

relative configuration. Meanwhile, as we reduced 16 to 21, performed periodate oxidation and obtained oxidation products, we were able to confirm the presence of α-ketol but the IR of the products revealed no carbonyl absorption at all. This suggested that 22 formed acetal rings with both the already existing carbonyl and with the newly produced carbonyl. If 20 is treated similarly it yields the same acetal 24 via 23. If the aforementioned 22 is acetylized it matches 24, so it was ascertained that no first-class hydroxyl groups took part in the formation of this acetal ring (Fig. 11). Since one obtains 25, 26 when dehydrating 20, the carbon adjacent to the third-class hydroxyl group is methylene and if one considers jointly the results of the decoupling of 24, one may estimate a partial a.b.c. structure, and the two-dimensional structure of 16 was derived[36]. Actually, since oxidized 16 becomes 17, and since 15 is obtained when this 17 is treated with a base, these structures were ascertained. At exactly the same time Barash et al isolated from Stemphylium botryosum stemphyloxin I (27) which resembles 17 and confirmed its structure through X-ray crystallographic analysis[37]. Later they isolated also stemphyloxin II (28) which corresponds to 15[38] (Fig. 12).

The structure of the other related compound 18 was determined in connection with that of 16[34] (Fig. 13). We first tosylated the first-class hydroxyl groups of 18, changed to 29 and obtained 30 through reduction of the same. Meanwhile, after having similarly changed 16 into 31, we reduced and obtained the same 30. The conclusion is that 18 is the hydroxylized C-21 form of 16. The structure of 19 was determined through various spectral analyses and that of 20 by relating to the derivatives of 16.

We reached the following conclusions with regard to the absolute configuration of betaenones. It seems from the ^{1}H NMR bonding constant that the B ring of 16 adopts a chair-form conformation. Under high-degree dilution of 20, IR indicates the intramolecular hydrogen bond (3480 cm^{-1}) originating from the C-2 hydroxyl group, and the A ring seems to take a three-dimensional conformation close to the twisted-type cyclohexane ring. It is estimated that because of this three-dimensional conformation there is not only a hydrogen-bond-based stabilization but that the bulky C-3 sec-buthyl groups and the C-4 methyl groups change into pseudoaxials from the axial which has great three-dimensional obstruction and that they become more stabilized. If one makes an octant projection of this three-dimensional conformation, a similarity to 16a is obtained and a positive Cotton effect is anticipated[33]. Actually, the ORD, CD curve of 16 shows a positive Cotton effect (Fig. 14) and the absolute configuration matches 16. At this time, the CD of 21 shows a weak negative Cotton effect, so it seems that the participation of the ketone of the C-4-position side chain is minimal. To ascertain further the absolute configuration we performed the periodate oxidation of 18 and induced the obtained 32 to the dibenzoate 33. We applied to this the exciter chirality method. As expected (33a), a negative Cotton effect was observed (Fig. 15) and this matched the previously obtained conclusion[39].

p. 362

From its structure, one supposes that betaenone B (16) is synthesized through the polyketide* route but there are two possibilities in the origination of the methyl groups branching off from the polyketomethylene chain. One is origination from the "C_1-pool" inside the living body (route a) such as methionine and the other is origination from propionic acid (route b)

(Fig. 16). With the purpose of clarifying this point we performed the incorporation of [1-^{13}C]-, [2-^{13}C]-, [1,2-^{13}C]-sodium acetate and [CH_3-^{13}C]-L-methionine, and obtained the result seen in Fig. 16[40]. It was concluded that the advance followed the route whereby a synthesis is performed from 8 acetic acid atoms and 5 methionine atoms, and this coincided with the biosynthesis route expected in most molds.

5.1.2 The aphidecholins Aphidecholin (34) was at first a compound isolated as a substance having an antiviral activity and a cell-division-inhibiting activity[41]. It was later isolated as a metabolite of Nigrospora sphaerica which attacks stored wheat[42] and as a plant growth inhibitor produced by Harziella entomophila[43]. It was later discovered that 34 inhibited specifically the eucaryote DNA polymerase α[44] and the same action was observed in the new related compounds 35, 36[45]. Recently 34 was shown to be extremely effective[46] in the cell division synchronization of cells in a plant suspension culture[46]. The characteristic of this synchronization is that no adverse effect is seen with regard to the genes encountered by other means. These spectral data of the structures of the compounds 35, 36, 37 related to the isolated aphidecholin show a similarity to those of 34 and estimation was easy. More especially with regard to 35, we were able to confirm the structure through derivation from 34 (Fig. 17). Recently Ono et al synthesized 35 expertly with l-abietic acid[47].

5.2 Altiloxin A (38), B (39) These are metabolic products of Phoma asparagi which attacks the asparagus stem[48,49]. The first example appeared as the driman-type sesquiterpene phytotoxin. Phoma asparagi is originally a temperate region disease but has recently become a frequently occurring p. 363

serious disease in the coastal areas of southern and mid-Hokkaido. The symptoms of the asparagus contracting the present disease is first the formation of light yellow or white fusiform lesions on the stem, followed by an red-brown blight of the entire stem. We still-cultured the agent at 26°C for 18 days in a potato broth, and after filtering and concentrating the culture solution we performed ethylacetate extraction. We fractionated the extracts with a silica gel column and submitted the various fractions to lettuce-seed-based biological examination. For the active fractions, we obtained the mixtures 38, 39, converted to methyl esters (40, 41) for separation, and after performing column fractionation we obtained the pure products 38, 39 via hydrolysis[48]. On the basis of HR-MS 40 is expressed as $C_{16}H_{26}O_4$, and 41 as $C_{16}H_{25}O_4Cl$, so we estimated with regard to 41 that one hydrogen atom of 40 had been replaced by chloride. We made a detailed analysis of the ^1H NMR of 41 and estimated the partial structures (a,b,c,d) shown in Fig. 18. Considering jointly the measurement results of the hydrogen bonds based on the chemical response, biosynthesis and IR, we estimated the two-dimensional structure of 41. To confirm this structure and the relative configuration we used the NOE difference spectrum. That is, when irradiating 15-CH_3 the 1-Heq, 9-H, 12-CH_3, 14-CH_3 signals increase and so do the 7-Hax' signals. So, we conceived for ring B a twist-boat shaped three-dimensional conformation containing α-epoxide. We confirmed the absolute configurations of 38, 39 by inducing these to the already known (−)-11-acetoxydriman-8-ol (43) (Fig. 19). To begin with, we converted 41 to 42 with dehalogenized hydrogen, induced this unto 40, and confirmed agreement with the natural methylester. We further converted 40 through 4 stages to 43 ($[\alpha]_D^{20}$ −8.2°), and confirmed this absolute configuration by comparing with the angle of optical rotation ($[\alpha]_D^{20}$ −9°C) given in the literature. p. 364

Keeping in mind the confirmation of the three-dimensional chemistry, the structure-activity correlation and the supply of altitoxin A (38) we performed the synthesis of 38[50] (Fig. 20). We first oxidized 44 by the 2-mode method and converted to 45. This 45 was further converted to olefin 46 through the 4 processes. When 46 was oxidized with m-chloroperbenzoic acid we obtained (±)-40(57%) and a small amount of three-dimensional isomers (10%). The three-dimensional selectivity of this oxidative reaction seems to originate from the β-side three-dimensional obstruction and from the 8-OH neighboring group effect. We hydrolyzed 40 and converted to altitoxin A ((±)-38).

Only low activity is shown by 38, 39 with regard to lettuce seeds and young plants but with regard to young asparagus plants which are the host, growth was inhibited by 50% in each case with 10 ppm. Recently, one synthesized the related compound 38 and examined the activity with regard to young rice plants. The results seem to indicate that epoxide is not essential to the manifestation of activity and that the hydrogen bonding between the carboxyl groups and the hydroxyl groups is involved in the activity.[51]

5.3 Solanapyrone A (47), B (48), C (49) and Zinnolide (52) Alternaria solani is an aggressive pathogen contaminating the solanaceae. The lesion caused by this agent exhibits round red-brown rings. The reported metabolic toxins are alternaric acid and a few more related substances[52]. One has recently pointed out the existence of host-specific toxins of undetermined structure[53]. We still-cultured the bacterium at 25°C for 25 days in a potato broth, condensed the medium filtrate under reduced pressure after which we extracted by chloroform and fractionated with a silica gel column.

We indexed growth-inhibition tests regarding lettuce seeds, and isolated 4 new phytotoxins which we called solanapyrone A (47), B (48), C (49)[54] and zinnolide* (52)[55]. The highest activity was shown by 47. Besides forming necrotic spots on potato leaves, a 100 ppm concentration inhibits to 100% the germination of rice seeds, and germicidity was indicated with regard to the Pericularia oryzae cavara pathogen.

The IR, UV spectra of 47, $C_{18}H_{22}O_4$ suggest the presence of α-pyrone rings. The substitution mode of this α-pyrone ring was from the shift value of ^1H, ^{13}C-NMR in virtual agreement with a (Fig. 21), so we judged that 47 contained such pyrone ring. Actually, this was corroborated by the fact that when 47 is hydrolyzed, one obtains ketoester 50. Since two-substitution-cis-olefin (δ5.44, 5.67, J=9.8Hz) is present in the residual hydrocarbon $C_{11}H_{17}$, the structure was estimated to be the 2-ring structure b. Furthermore, through decoupling tests 1-H was shown to be in a transdiaxial (J=11.7, 9.8 Hz) position relationship with 10-H, 2-H respectively, and from the J values (4 Hz) of 5-H, 10-H, the ring function was estimated to be cis.

We determined the absolute configuration of 47 by applying the CD exciter chirality method to this dibenzoate derivative 51[56]. Since the CD spectrum of 51 showed a negative first Cotton (Fig. 22), the absolute configuration of 47 was determined as (1R, 2S, 5R, 10R).

The spectral data of solanapyrone B (48), $C_{18}H_{24}O_4$ are similar to those of 47 but from ^1H or ^{13}C NMR there are no formyl groups and as hydroxyl group absorption is observed in the IR, 48 was estimated to be a structure whereby the formyl groups of 47 had become hydroxymethyl groups. Actually, 47 was produced through the oxidation of 48.

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The spectrum of solanapyrone C (49), $C_{19}H_{25}NO_4$ resembles that of 47 but the fact of a nitrogen-holding compound was indicated. Instead of the methoxy group, we noted in the ^1H NMR the presence of 2 methylene atoms and monoacetate is formed through acetylation. This is why 49 was judged to be a structure in which ethanolamine groups had been substituted to the pyrone ring. Actually, 49a is formed by the structure shown in Fig. 23 when 49 is hydrolyzed, and the structure was corroborated. p. 365

On the basis of the spectral data, zinnolide (52), $C_{15}H_{18}O_5$ was judged to be 5-substitution benzene containing prehnylether. The reduction products based on the $LiAlH_4$ of 52 agreed with the already known compound Zinniol (54) isolated from Alternaria zinniae (54), so the compound seemed to be either 52 or 53. Meanwhile, when comparing the reduction products based on the $NaBH_4$ of 52 with the two phthalides 55, 56 obtained through the oxidation of 54, there was agreement with 55, so the structure of zinnolide* was determined to be 52[55] (Fig. 24).

Considering the structure confirmation and the development of a supply method, we used the most biologically active solanapyrone A (47) as labelled compound, and performed the synthesis hereunder[57] (Fig. 25). To synthesize we used the method which builds at once the decalin skeleton from the intramolecular Diels-Alder reaction of the triene (61). We first derived the pyrone portion 58 in several steps from dehydroacetic acid (57). Meanwhile we used sorbic acid as starting material and obtained aldehyde 60 through the acetate 59. We condensed the pyrone derivative 58 and the aldol of (E,E)-dienal 60, and obtained the (E,E,E)-triene 61 through three-dimensional selection. When this is heated for 1 h at 180°C in toluene, one obtains the three-dimensional isomer mixtures 62, 63 at the 1:2 rate. Regarding this production

ratio, no improvement was seen either with the other solvents. When reacting at below 180°C the mixture (E,E,E)-61 + (E,Z,E)-61 (1:1) derived by another route from crotylbromide, only (E,E,E)-61 reacted, and 62, 63 were yielded. p. 366 However, no additional substance was obtained from (E,Z,E)-61. Similar results were observed in the synthesis of diplodiatoxin[58] and this is noteworthy as an example of dynamic control of the intramolecular Diels-Alder reaction. Finally, we hydrolyzed this three-dimensional isomer mixture (62, 63) and isolated solanapyrone A (±)-47 and its three-dimensional isomer 64 in a 2:3 proportion.

5.4 Alboatrin (65) Since the use of germicides is generally limited with regard to pasture grass blight, the breeding of resistant varieties has become essential. As alfalfa is a good leguminous pasture grass it is an important grass species. However, the verticillium wilting disease which is soil-transmitted broke out and has become a factor of pasture degeneration. Since the traditional resistance inspection methods in which pathogens are inoculated is not necessarily effective, it has become necessary to develop simple inspection methods using toxins and such. We performed from this viewpoint the examination of the phytotoxin produced by Verticillium alboatrum which is the pathogen of the present disease. We first still-cultured the agent at 22°C for 40 days in a potato broth medium, extracted after concentrating the culture solution and fractionated by chromatography. We indexed alfalfa growth inhibition tests with regard to the various fractions, and obtained 3 toxic substances[59]. These are the already known orcinol, orcinolmonomethylether and the new compound 65 called alboatrin after the scientific name[59] (Fig. 26). The molecular formula $C_{14}H_{18}O_3$ expresses 65.

The monoacetate shows no hydroxyl absorption in the IR. It is therefore believed that the 2 remaining oxygen atoms have an ether bond. Since the a·b·c partial structures are conceived from ^{1}H NMR (Fig. 26), we assumed the structure 65 by combining the same. By measuring the NOE difference spectrum we were able to ascertain that this structure contained a relative configuration. The structure of 65 is somewhat singular for a natural product but understandable if we consider the complex biogenesis route as shown in Fig. 27. This means that orcinol is first produced by the polyketide route and that after a nucleophylic reaction with dimethylallylpyrophosphate, it becomes 65 via an intramolecular reaction.

We performed the synthesis of 65 so as to ascertain the structure (Fig. 28). Reducing the already known ester 66, we converted to the alcohol 67 and by the Friedel-Crafts reaction changed this to 68 even if only at a low yield rate. As anticipated, the hydrogen addition in the acetic acid 68 gives at once alboatrin (65) as a unique product and did not produce the 3 other possible three-dimensional isomers. The reason is that the 3-substitution double bond of the fran ring 68 undergoes the reduction preferentially and becomes the intermediate 68a. This is because it is immediately converted to 65 via the antiaddition which is three-dimensionally desirable. Using a column for optical isomer separation we performed the optical separation of (±)-65 into (+)-65 (natural type) and (-)-65.

We demonstrated that at a low concentration the physiological activity of (+)-65 prevents more the growth of alfalfa seeds than that of lettuce seeds, and a comparison with orcinol and orcinolmonomethylether demonstrated a high growth-inhibition activity. Furthermore, among the varieties of alfalfa, a high growth inhibition activity is shown more clearly with regard

to the resistant varieties than with regard to the sensitive varieties. Further examinations are called for to distinguish the low-resistance varieties.

6. Conclusion

We outlined the chemistry of the toxins produced by phytopathogenic microorganisms. Because of the limitations of the present article, we did not touch on the topic of numerous other toxins. According to the recent reports the phytopathogens produce wide-ranging physiologically active substances such as the self-growth-inhibiting substance chloromonilycine*[60] of the cherry fruit ash star pathogen*, the toxins of the phytopathogens hosted by weeds[61], the karyokinetic inhibitor macrolide, lysoxin[62] obtained from the rice blight pathogen, and one might discover numerous useful substances. Meanwhile, p. 367 one is presently attempting practical use of the toxic action itself with the aim of growing low-resistance plants through the selection of cultured cells from the host plants[63]. It should be interesting to watch the future developments.

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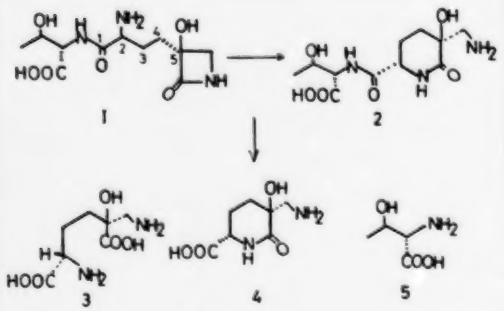
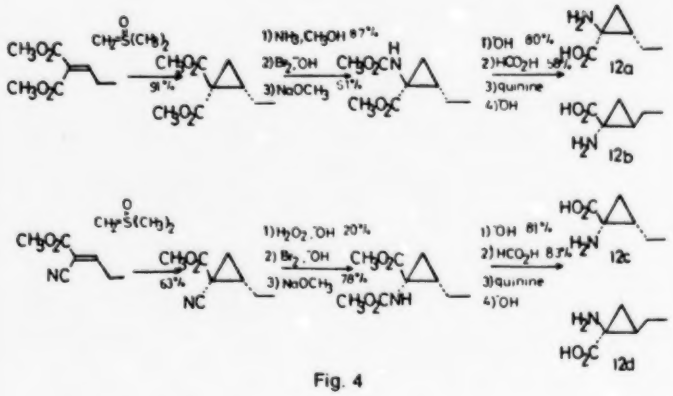


Fig. 1



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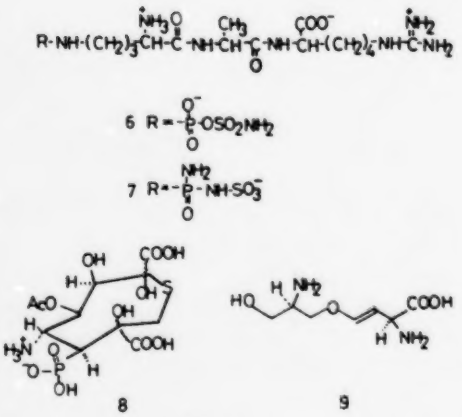

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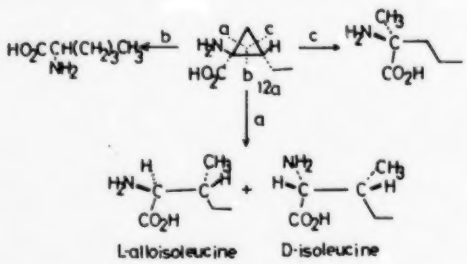
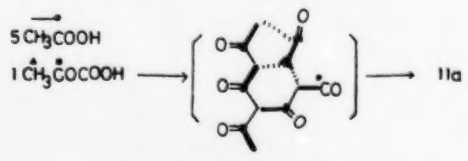


Fig. 5



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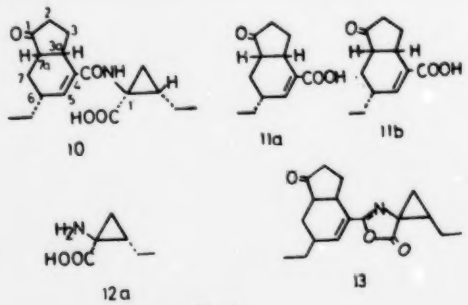

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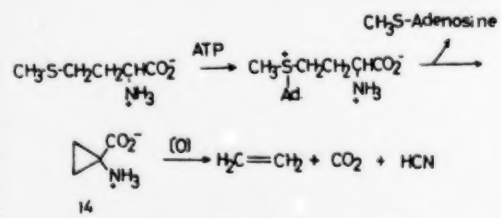

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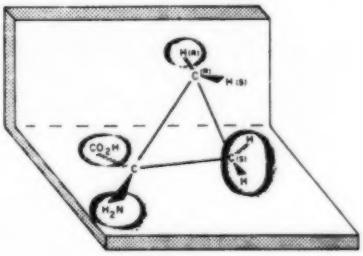

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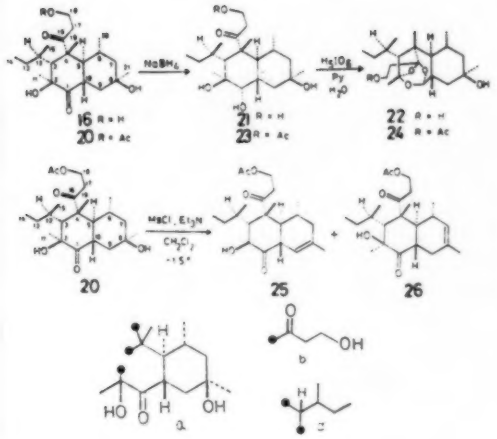

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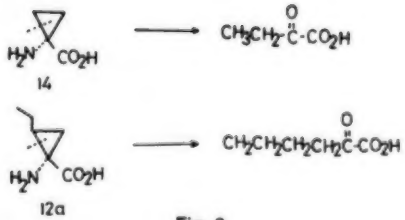

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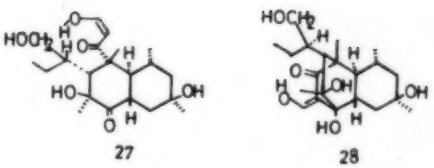

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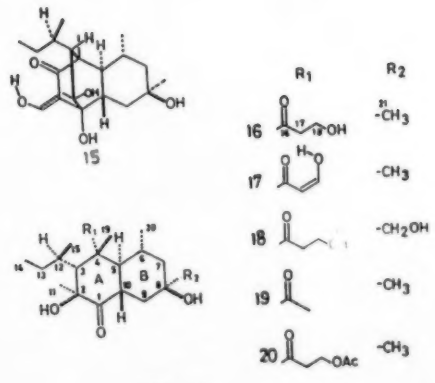

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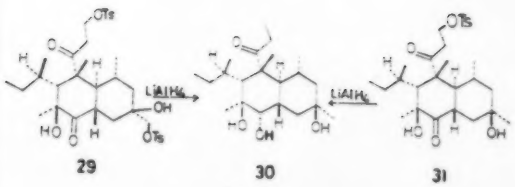

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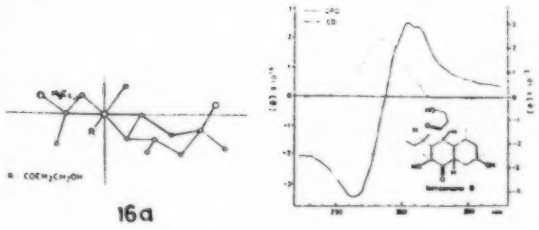
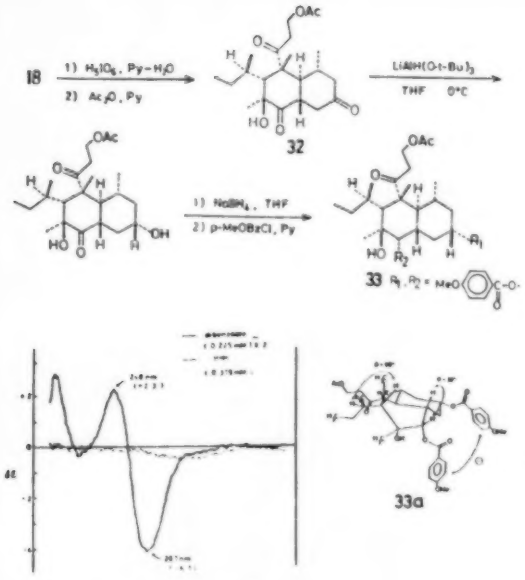


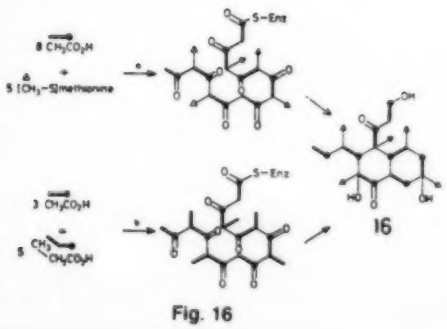
16a

Fig. 14



33a

Fig. 15



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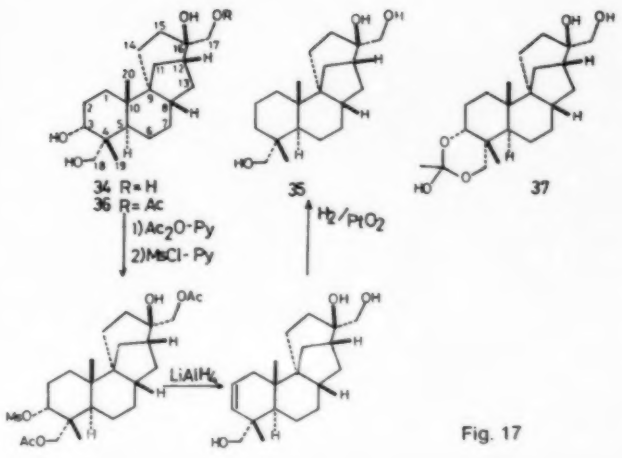

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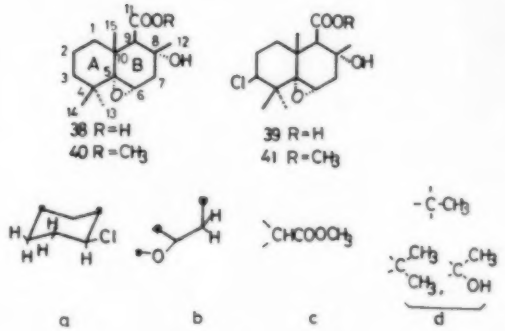

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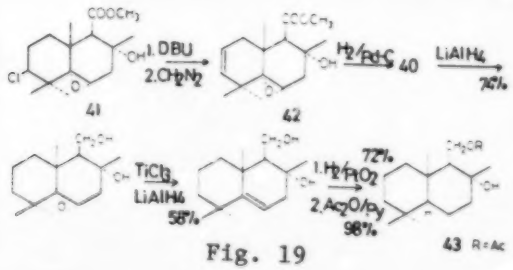

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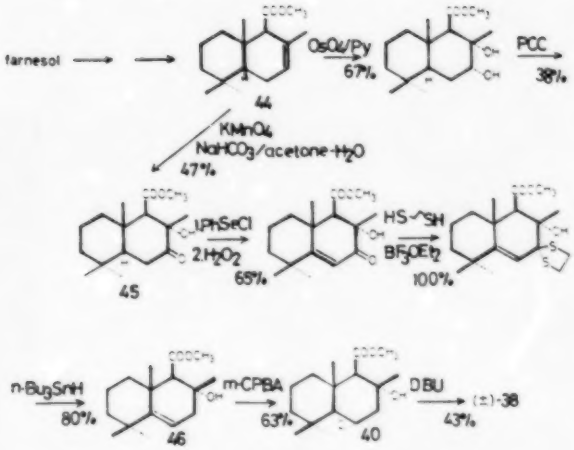

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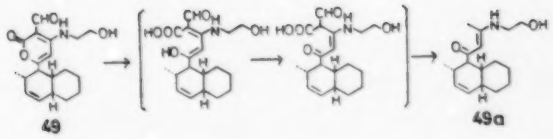

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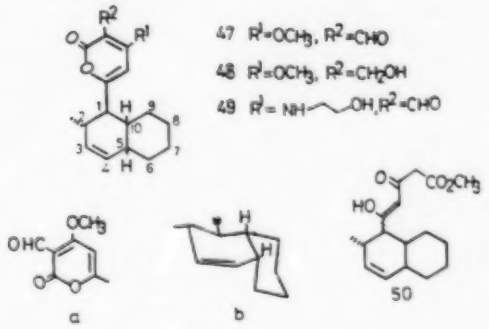

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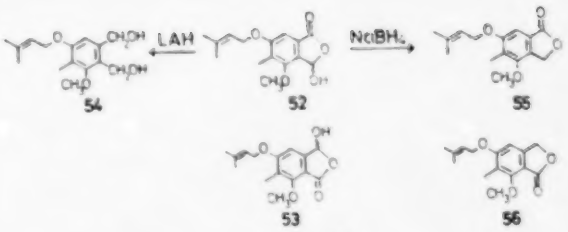

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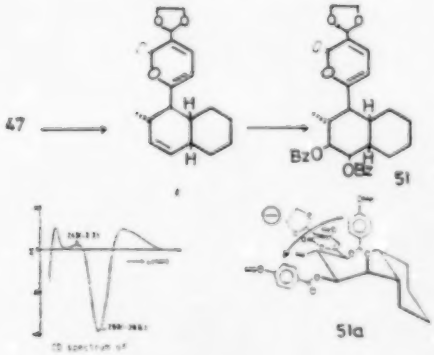

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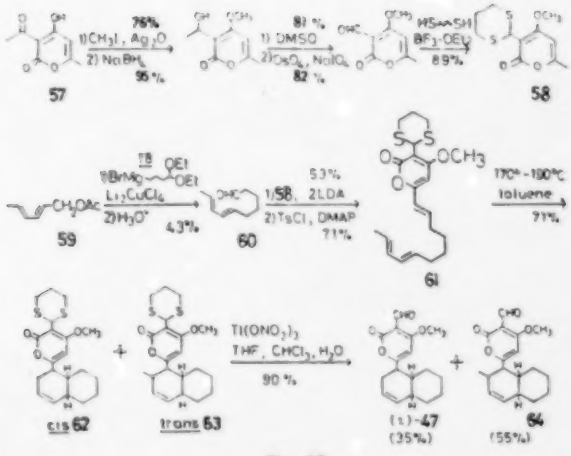
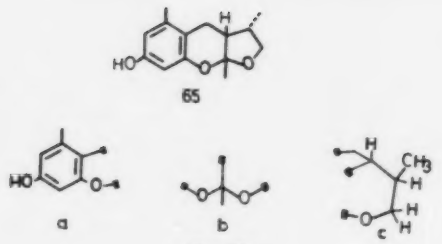


Fig. 25



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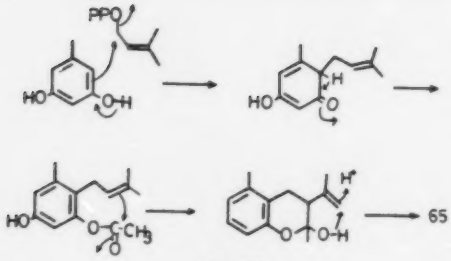

Fig. 27

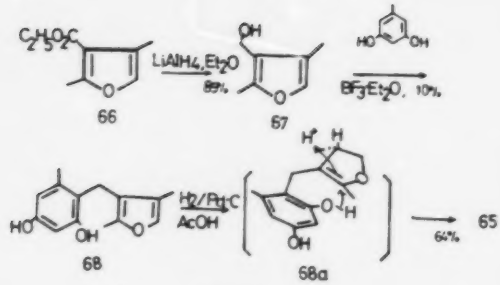

Fig. 28

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*Translator's note: Reading not quite certain; same hereafter.